LF Raman Spectroscopy

+ other stuff

G. Patrick Stahly, Ph.D.
pstahly@tricliniclabs.com
www.tricliniclabs.com

Triclinic Labs

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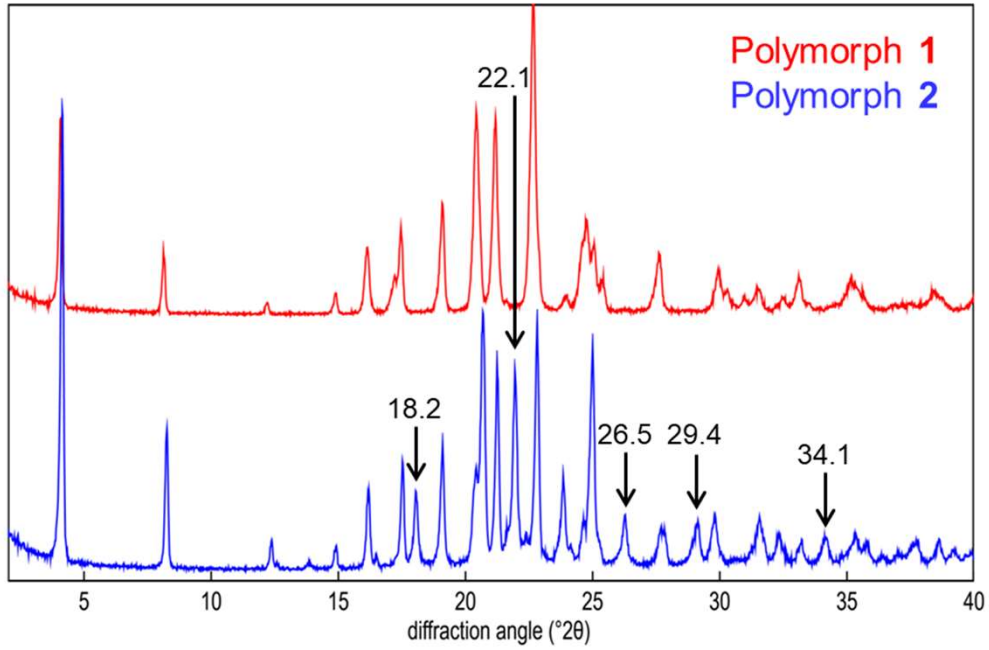
+ other stuff

This presentation describes a system of isoenergetic polymorphs (polymorphs that are “virtually indistinguishable except by means of X-ray diffraction”)[1]. Reported are analytical data from multiple techniques, quantitative XRPD method development, and the use of low-frequency (LF) Raman spectroscopy for both qualitative and quantitative analyses.

1. Carstensen, J. T.; Franchini, M. K. Drug Dev. Ind. Pharm. 1995, 21, 523-536

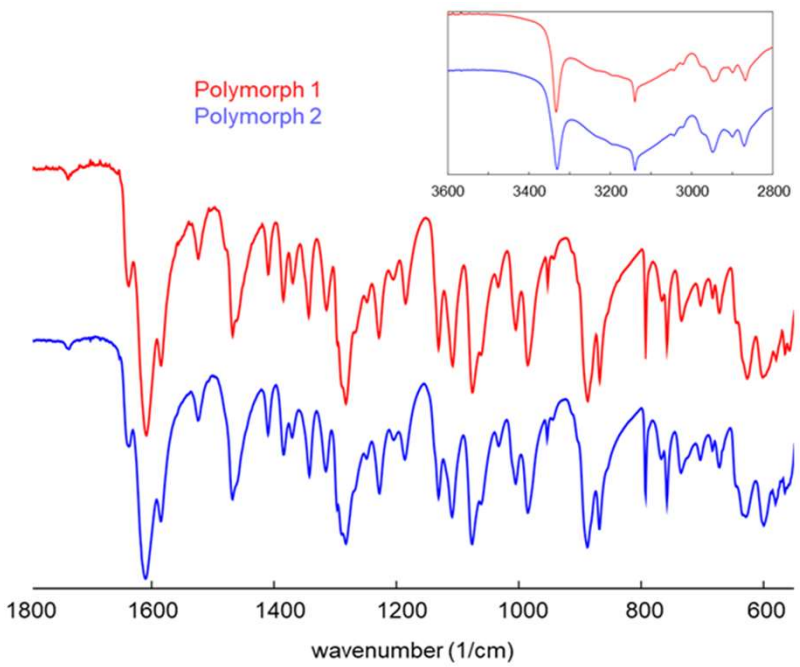
The System

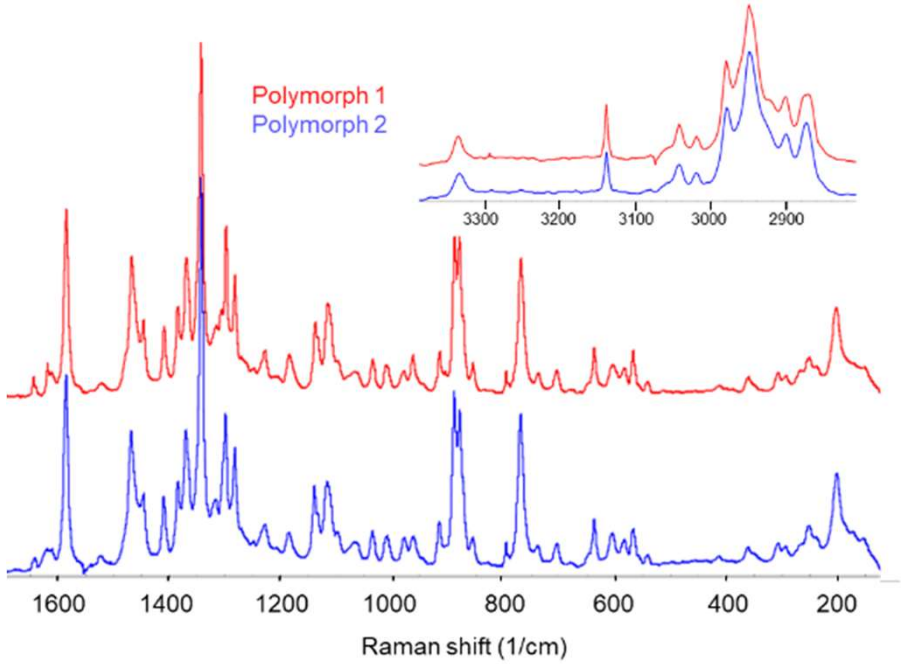
- An API that exists as two, isoenergetic anhydrates.
- Polymorph **2** contains several free-standing peaks in its XRPD pattern that are not in the pattern of polymorph **1** (arrows).
- The reverse is not true.
  - Difficult to distinguish pure **2** from mixtures of **1** and **2**.



The System

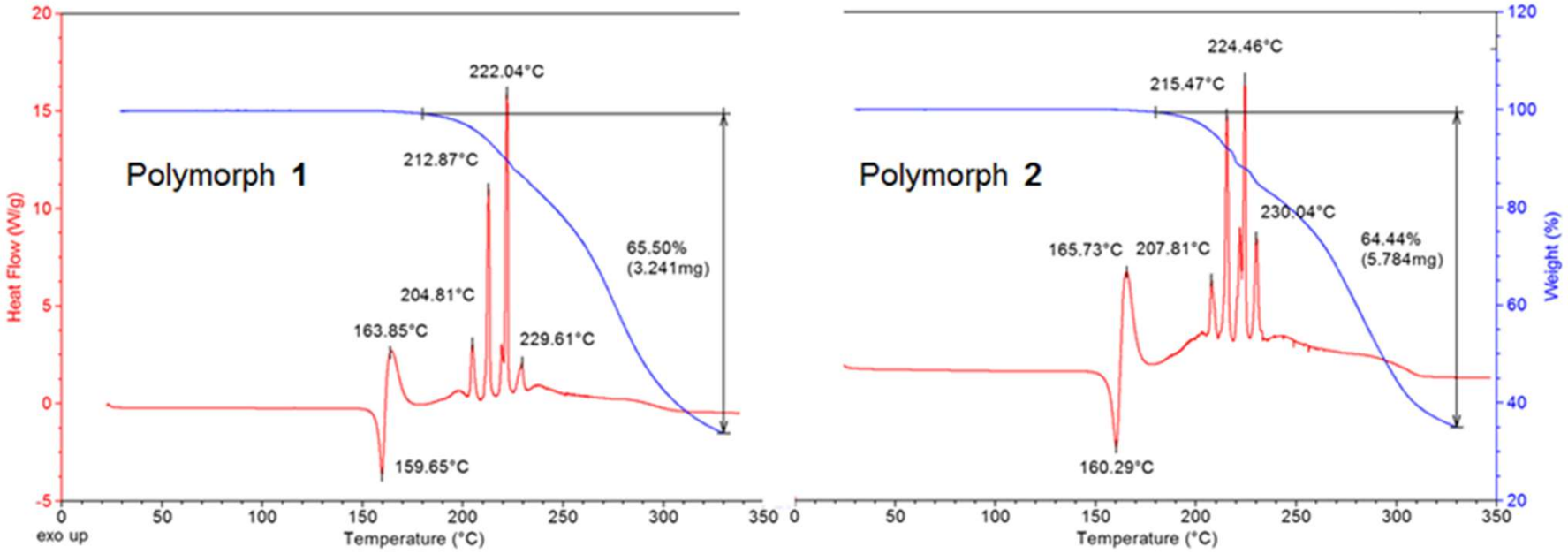
Vibrational Spectroscopy





The System

DSC and TG

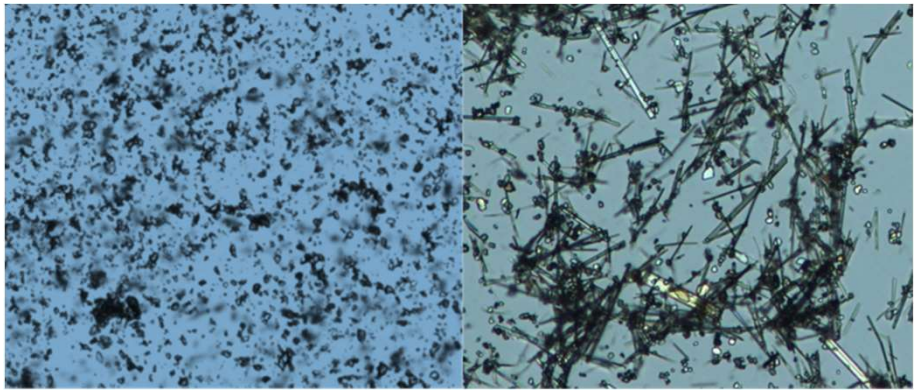


melt 160 → recrystallization 165 → decomposition 205 on

what's here?

The System

- The API was formulated as an aqueous slurry using micronized **1**.
- Over time, solids suspended in formulation vehicle changed appearance from featureless particles to plates and needles.

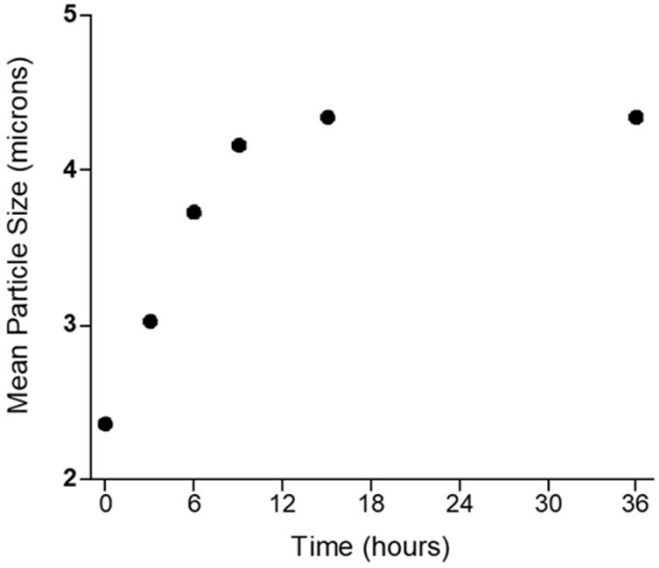


freshly-micronized API in DP

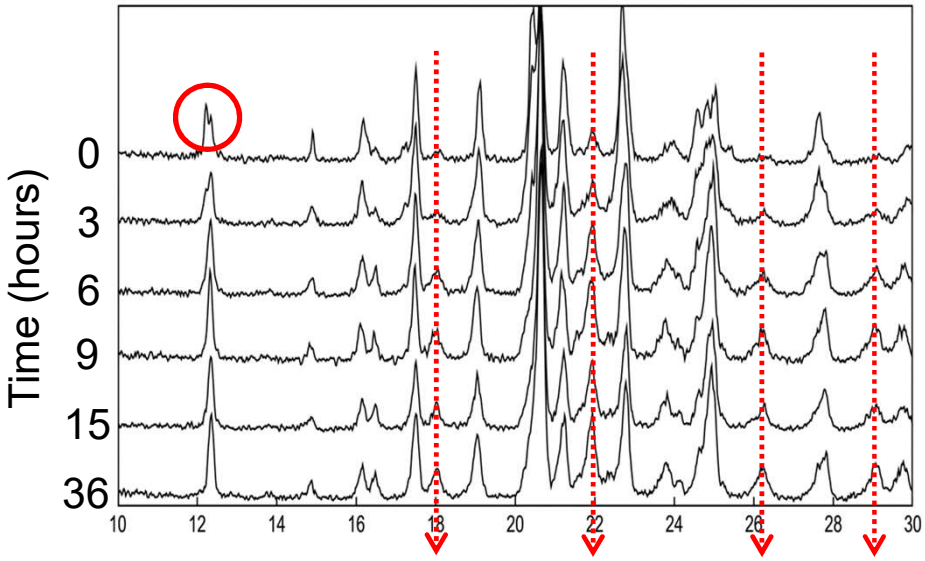
DP after 3 months at 5 C

- Questions
  - What is happening?
  - How to generate a stable drug product?
- Analytical methods are needed that differentiate **1** and **2**.

The System

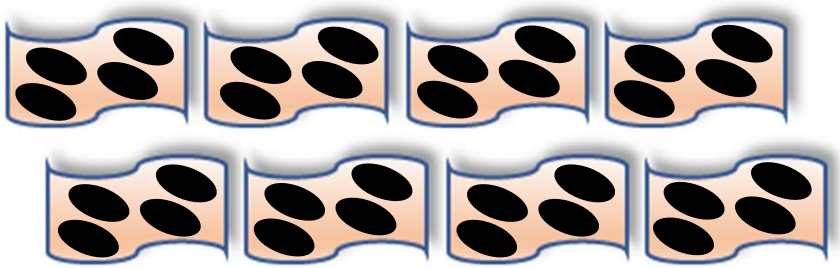


Plot of the d10 values obtained from a slurry of micronized API in an aqueous formulation vehicle at 40 ºC

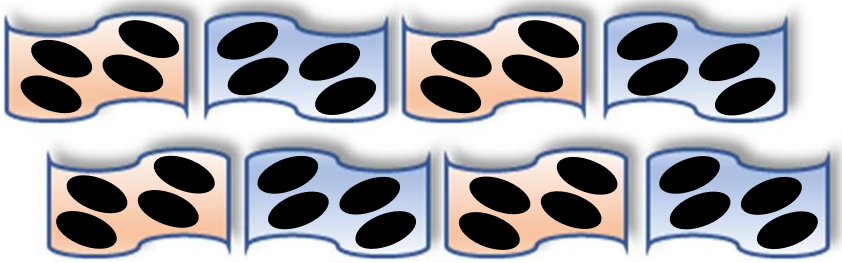


Crystal Structures

Polymorph 1



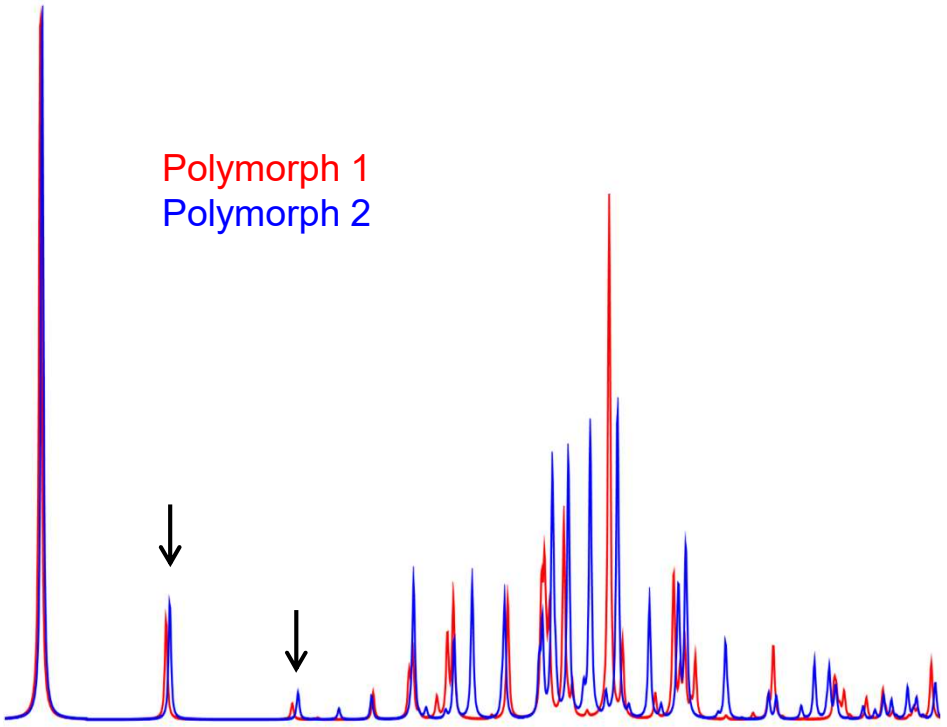
Polymorph 2



c axis

	Polymorph **1**	Polymorph **2**
crystal habit	plate	needle
crystal system, space group	monoclinic, $P2_1$	orthorhombic, $P2_12_12_1$
data collection temperature (K)	100	100
a (Å)	5.5022(6)	5.4710(11)
b (Å)	7.0634(6)	7.1450(14)
c (Å)	21.5819(14)	42.333(8)
α (°)	90	90
β (°)	95.621(6)	90
γ (°)	90	90
volume (Å^3)	834.73(3)	1654.8(6)
Z	2	4
d_{calc} (g cm^{-3})	1.513	1.527
R factor (%)	3.9	3.6

Crystal Structures



small but measurable differences in the diffraction maxima from a specific set of (h,k,l) planes

XRPD Method

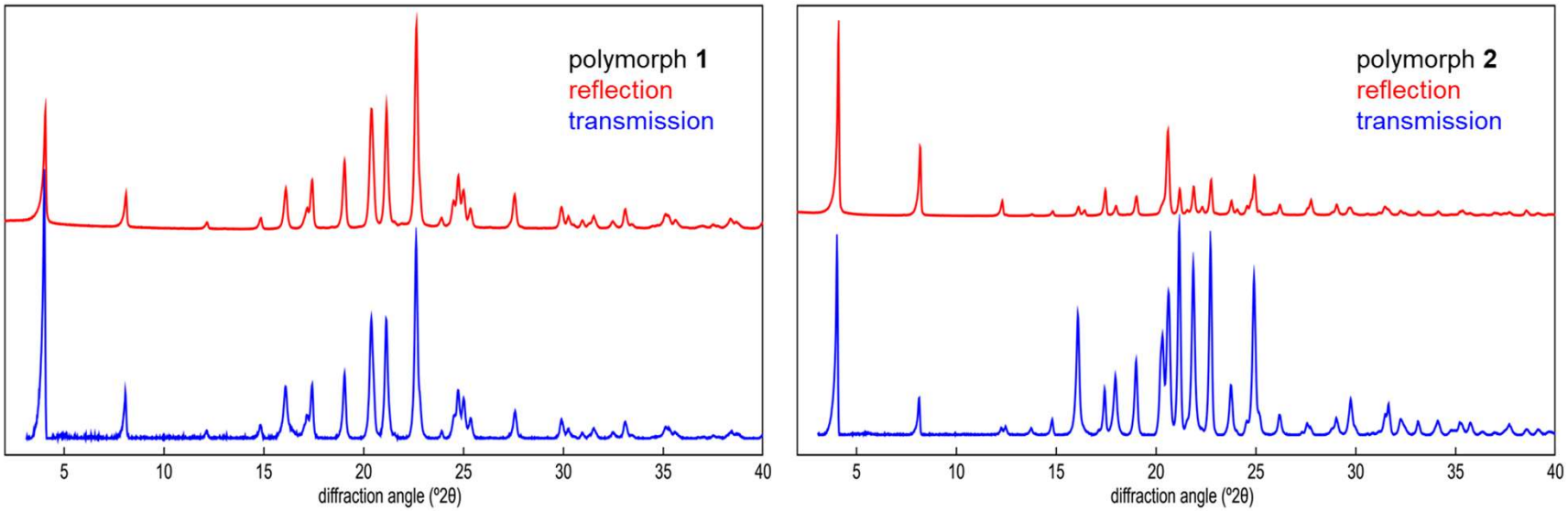
- Careful examination of XRPD patterns (previous slide) suggestes they can be differenatiated by full-pattern analysis
- Triclinic routinely develops solid mixture analysis methods using XRPD
- Using XRPD for quantitative (or semiquantitative) measurements presents problems that do not occur when using liquid techniques such as HPLC

    - mixing of standards
    - preferred orientation effects
    - sample/specimen size
    - minimum balance weight
    - non-sample data
    - minor specimen offset
    - changing tube power

XRPD Method

- Mixing of standards
  - Solids do not mix homogeneously
    - avoid sub-sampling
    - irradiate entire mixture
  - In this case it is difficult to distinguish pure **2** from mixtures of **1** and **2**
    - how can mixtures be made containing known amounts of **1** and **2**?
- Preffered orientation effects
  - Random changes in relative peak intensities render standard plots of peak heights or areas against known concentrations useless
  - In this case XRPD patterns of polymorph **2** clearly exhibit the effects of preferred orientation

XRPD Method



XRPD pattern of P2 is affected by preferred orientation

XRPD pattern of P1 is not

XRPD Method

- Sample/specimen size
  - The sizes of specimen holders for XRPD diffrctometers are designed so that as much as possible of the specimen is irradiated by the incoming X-rays
    - specimen holders are small in all three dimensions
    - reflection holders used at Triclinic hold between 5 and 25 mg of material, depending on density

- Minimum balance weight
  - USP chapter 41: balances must be calibrated over their operating range and meet the requirements defined for repeatability and accuracy.
    - minimum weight of most sensitive balance at Triclinic (6-place) is about 1 mg

XRPD Method

Here is a real-world example of the effect of the combination of specimen holder size and minimum balance weight:

- Triclinic developed an XRPD method that had a detection limit of about 0.2% crystalline material in an amorphous matrix, determined based on signal-to-noise ratios
- To confirm that detection limit a mixture of 0.1% crystalline in amorphous was required
- The minimum weight requirement (1 mg of crystalline material) limited the total sample weight to no less than 1 gram
- Only 8 mg of the very fluffy mixture fit in the specimen holder
- Each XRPD measurement took 65 minutes at the optimum measurement speed (0.2 °2θ/min)
- It would take 125 specimens to analyze a single 0.1% mixture
- Data from multiple aliquots were co-added to give a final data set for each mixture
- XRPD sensitivity depends on counting (Poisson) statistics, so signal-to-noise = $N/\sqrt{N}$
- The desired sensitivity is reached with a 65-minute scan time of a single sample. The same sensitivity can be obtained by analyzing each of the 125 aliquots for only 31 seconds each (65 min ÷ 125). Although the diffractometer time is the same no matter how many aliquots are analyzed, a larger number of aliquots means more analyst time is required to prepare specimens.

XRPD Method

- Non-sample data
  - X-rays reaching the detector can arise from things other than the sample
    - incoherent scattering by air (the scattered radiation is of a different wavelength than the impinging radiation)
    - Compton scattering (incoherent scattering from specimen)
    - Bremsstrahlung radiation (continuous wavelengths from the tube)
  - Pre-processing XRPD data is necessary
    - collect data from an empty specimen holder and subtract it from specimen data
    - digital filtration
    - smoothing
    - normalization

XRPD Method

- Minor specimen offset
  - Arises from specimen surface not being exactly at diffractometer focal plane
  - Requires pattern shifting to common peak position with standard
- Changing tube power
  - X-ray tubes decay with time
  - Requires setting tube intensity limits and monitoring as part of the method to insure DL is still valid
    - Attenuate tube to approximate intensity loss & test DL mixture
    - Calculate using signal-to-noise data

XRPD Method

- Problems to overcome
  - The polymorphic purities of reference standards are unknown
  - Therefore, mixtures of known concentrations cannot be made
  - XRPD patterns from P2 are severely affected by preferred orientation

- Data collection
  - multiple spectra from samples containing mixtures of unknown concentrations

- Data Analysis
  - smooth
  - filter
  - normalize
  - pattern shift
  - principal component analysis (PCA)
  - alternating least squares (ALS) analysis

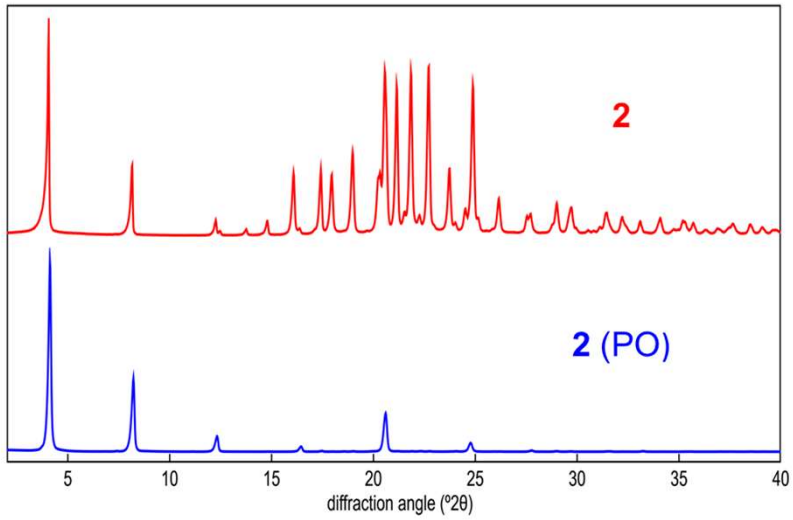
XRPD Method

- A ‘standardless’ XRPD method was developed
  - To overcome the issue with preparation of physical standards, standard XRPD patterns were identified
    - chemometric analyses starting with reference pattern from polymorph **1**
    - Rietveld analyses starting with the unit cells of **1** and **2**
    - principle component analysis limited to 2 components
    - widely variable numbers from the above analyses indicated that the patterns cannot be described by a simple binary system (PO effect)

    - principle component analysis limited to 3 components identified 3 reference patterns that represented all mixture data

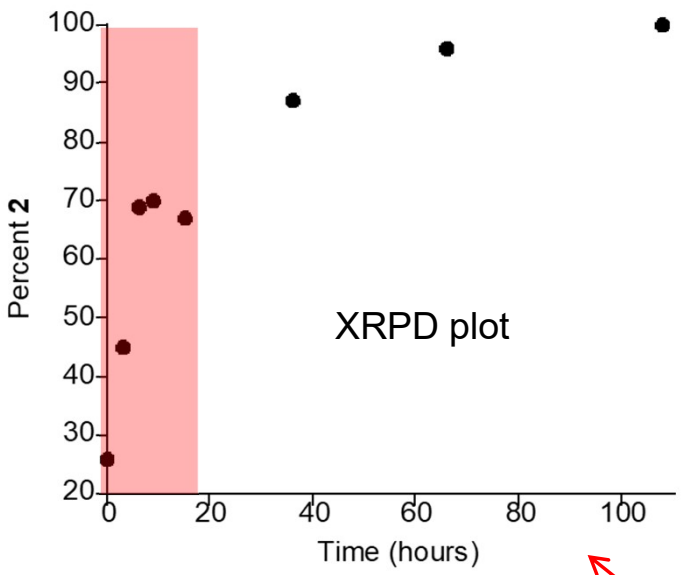
reference pattern 1 =
pattern measured from **1**

reference patterns 2 and 2(PO) →

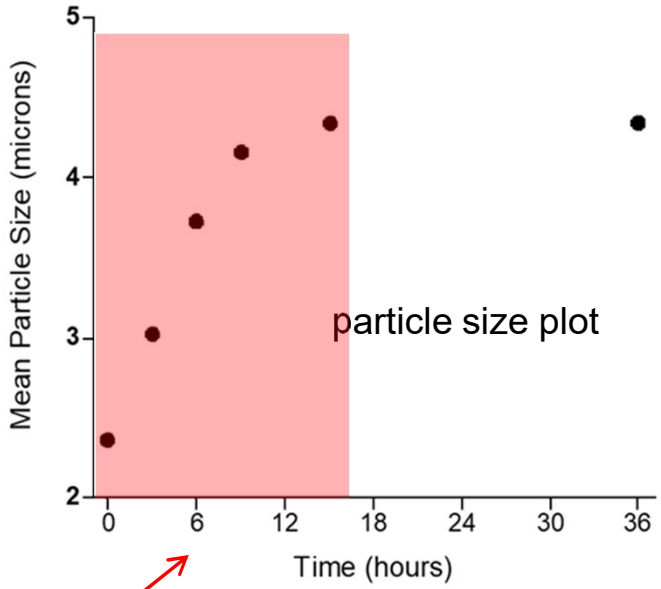


XRPD Method

A semi-quantitative method was developed using random-forest pattern clustering followed by alternating-least-squares analyses. That process is described as semi-quantitative because method validation was not carried out. For each measured pattern from a sample of unknown content, the best matching pattern was calculated by linear combinations of the three reference patterns. The amounts of each polymorph were then derived from the amounts of each reference pattern used. Note that the amounts of polymorph **2** were calculated by addition of the amounts of reference patterns 2 and 2(PO).



XRPD plot



particle size plot

note scale difference

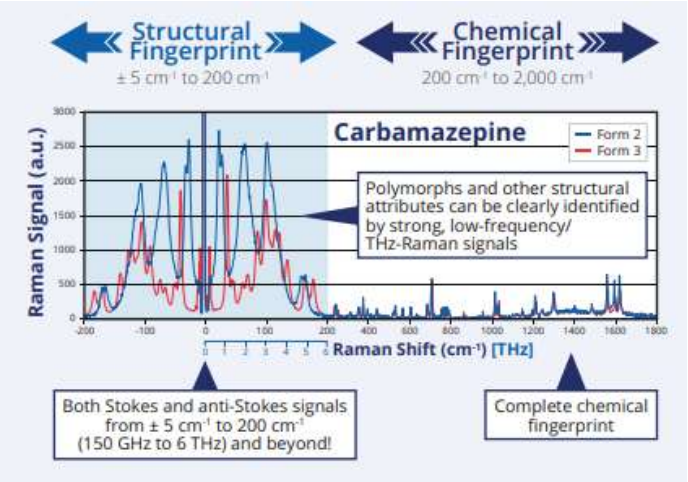
LF Raman Spectroscopy

Triclinic Labs acquired LF Raman capability during the isoenergetic polymorphs project

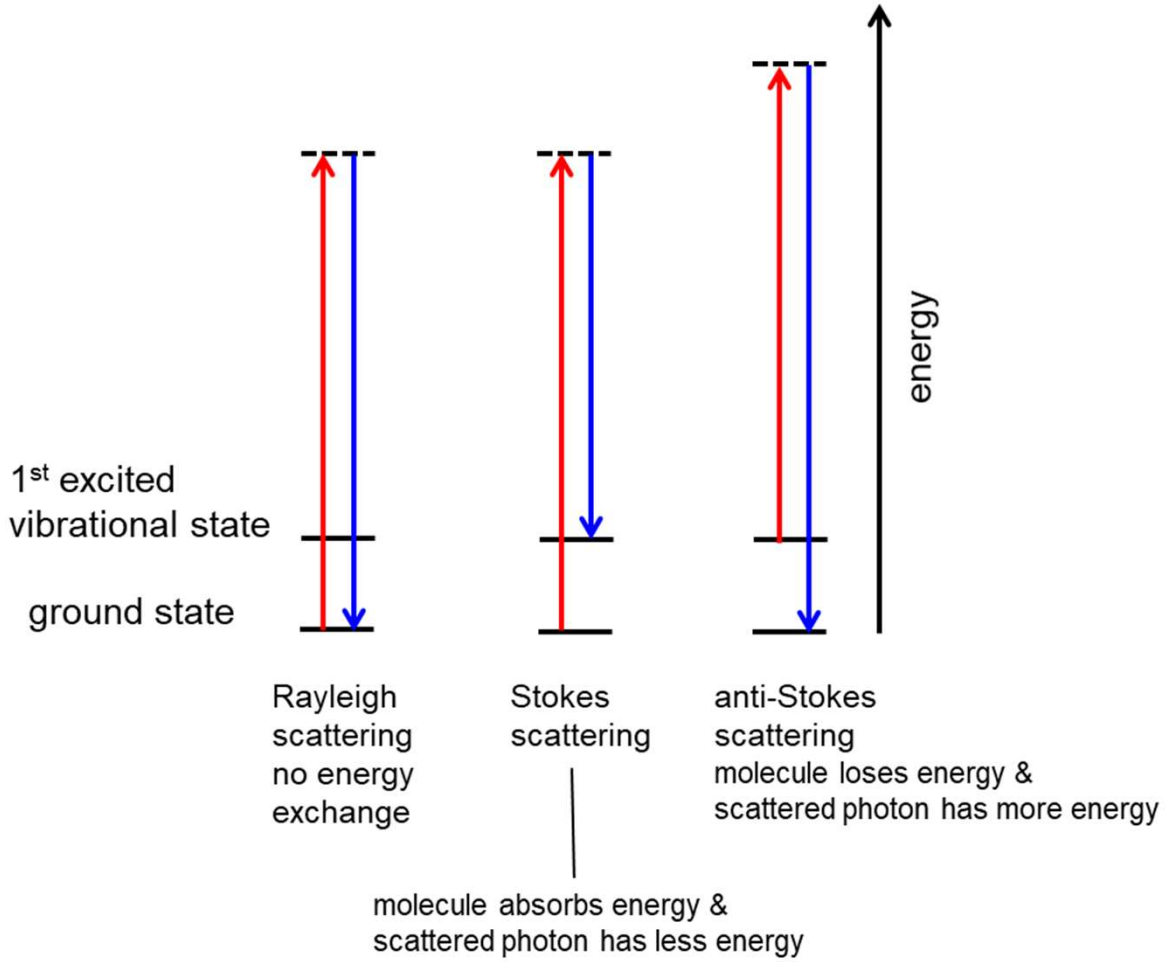
LF Raman Equipment

ONDAX (now COHERENT) SureBlock™ ultra narrow-band notch filter

Tablet holder and probe sample interfaces

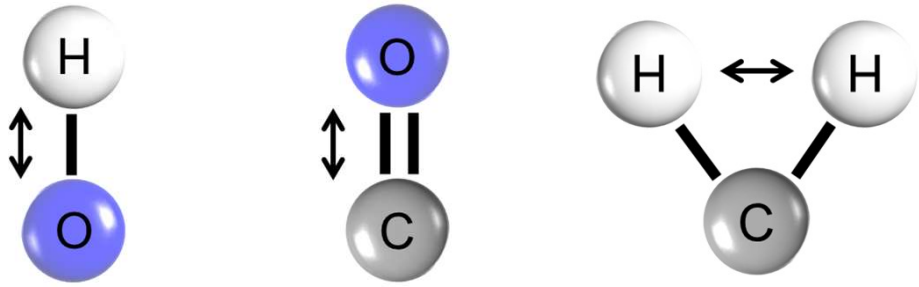


LF Raman Spectroscopy



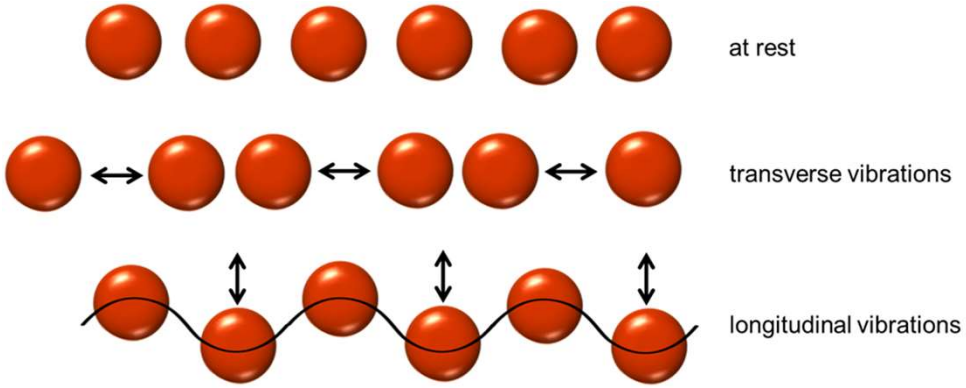
Phonon Vibrations

bond vibrations
500-4000 cm^{-1}

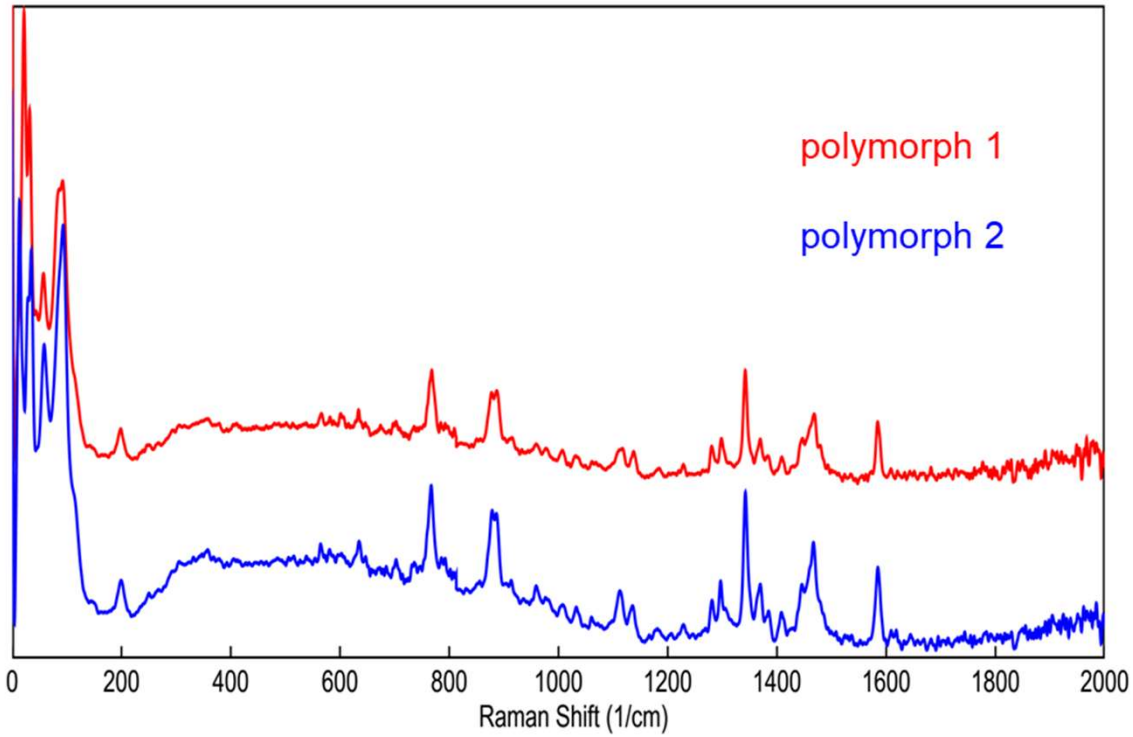


3400-3600 cm^{-1} 1550-1900 cm^{-1} 1430-1490 cm^{-1}

phonon vibrations
5-500 cm^{-1}

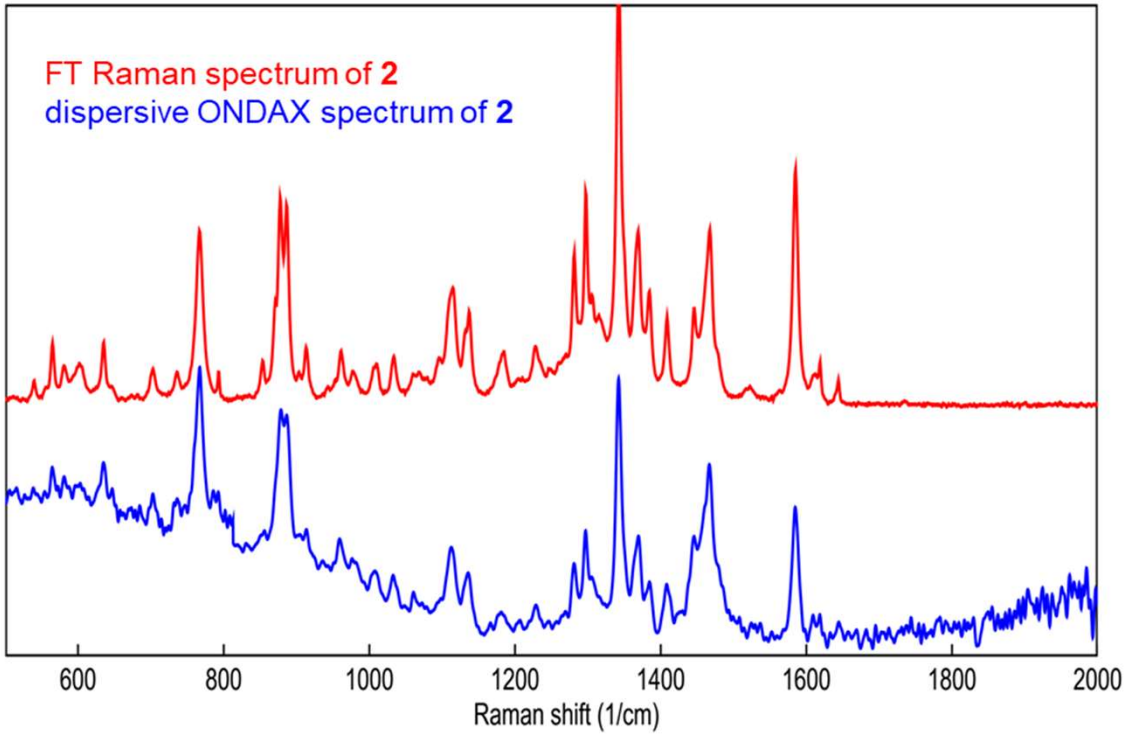


LF Raman



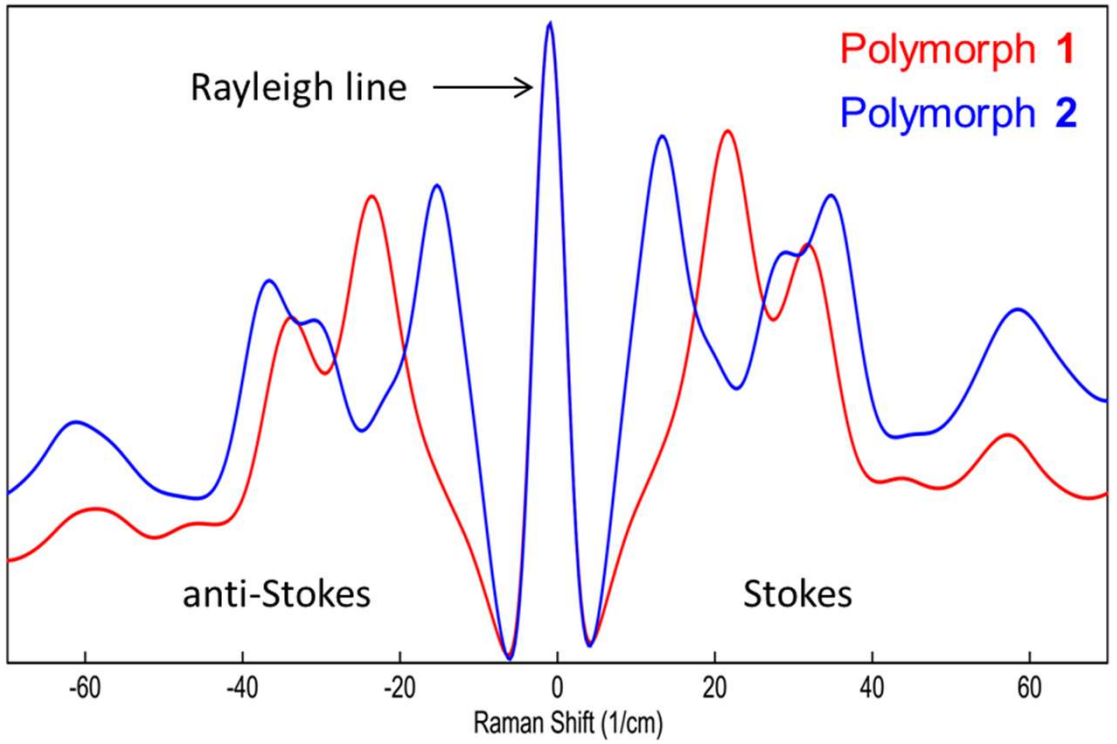
full Raman spectra acquired for each polymorph

LF Raman



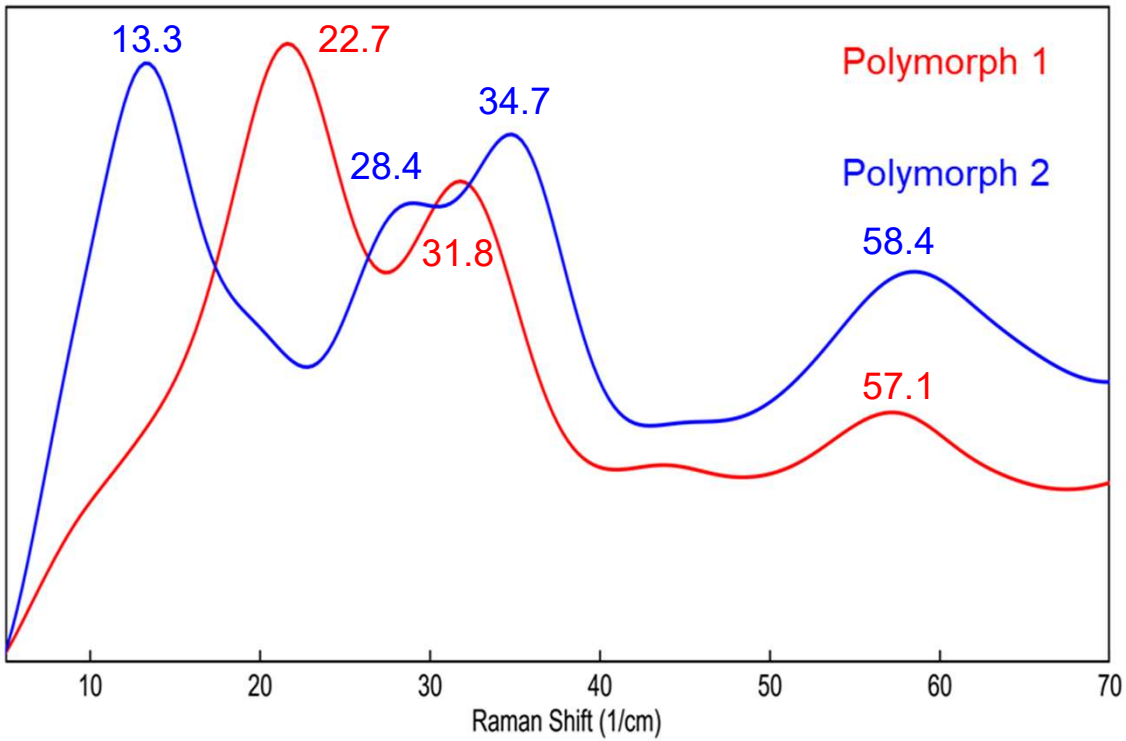
correlation between FT (1064 nm) and dispersive (853 nm) Raman spectra

LF Raman



differentiation of P**1** and P**2**
note Stokes more intense than anti-Stokes

LF Raman



differentiation of P**1** and P**2**
Stokes signals only

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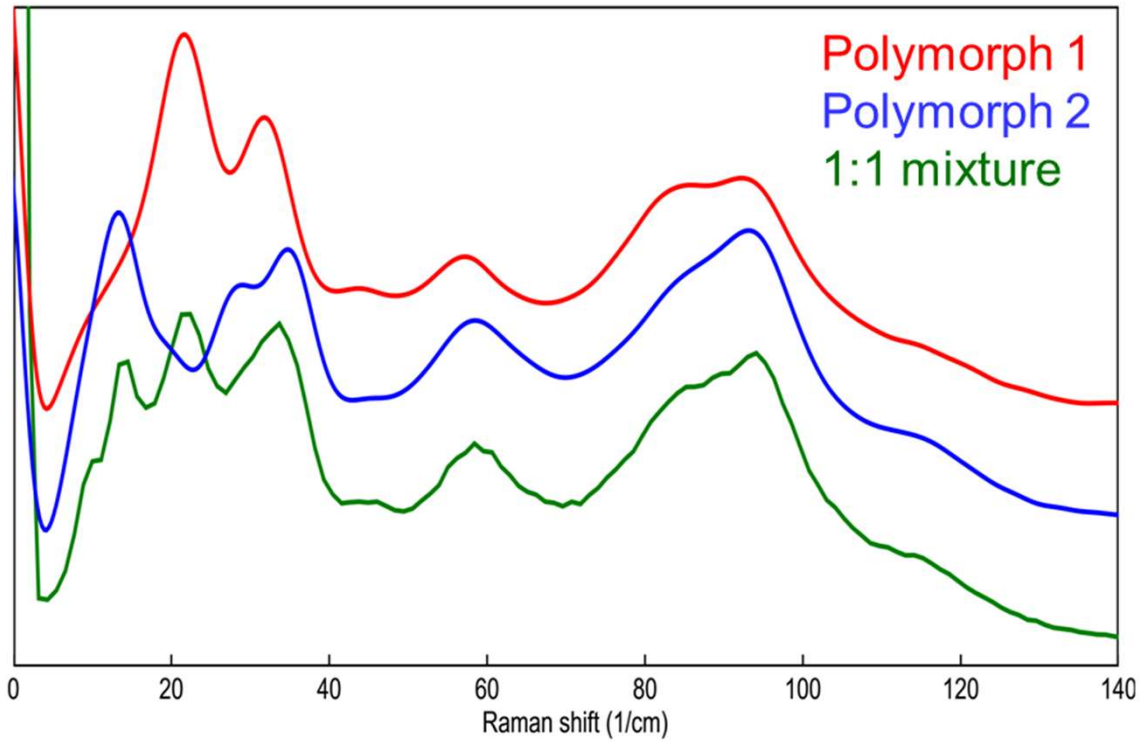
LF Raman



differentiation of P**1**, and P**2**, and mixture
Stokes signals only

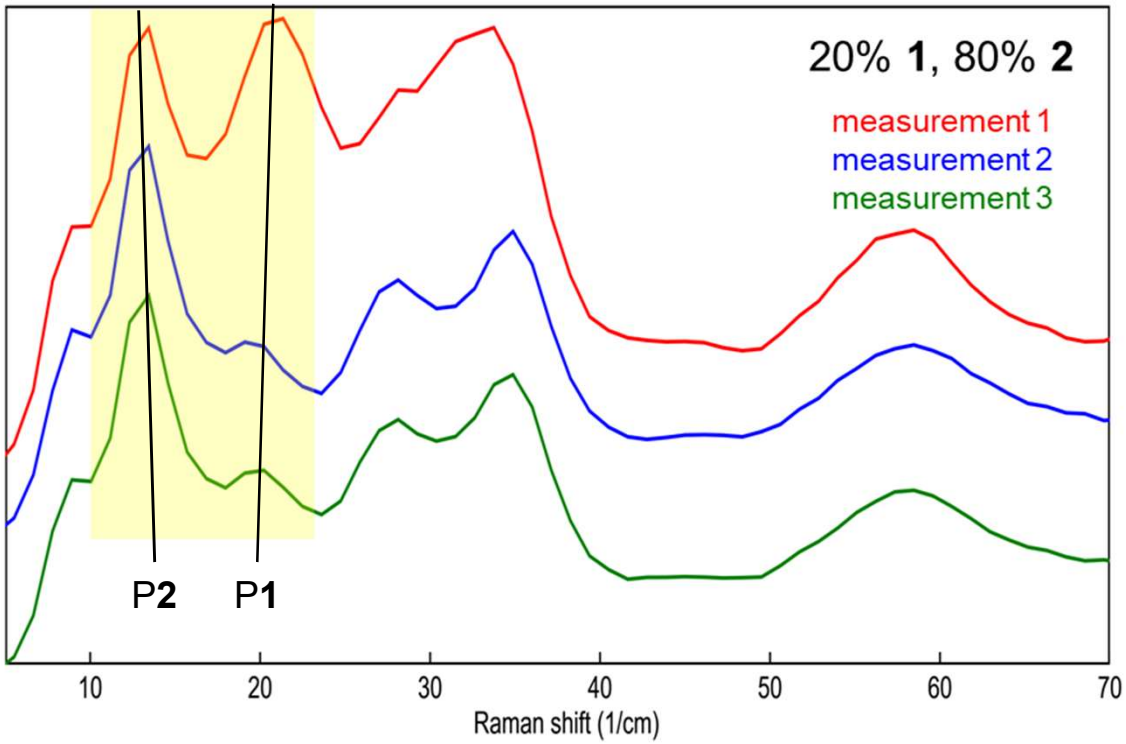
LF Raman

mixtures were made and analyzed

P1	P2
0	100
20	80
40	60
50	50
60	40
80	20
100	0

- multiple spectra were collected from each using the contact/immersion probe in contact mode
- mixtures are inhomogeneous (next slide)
- will try slurry of mixtures in inert liquid

LF Raman

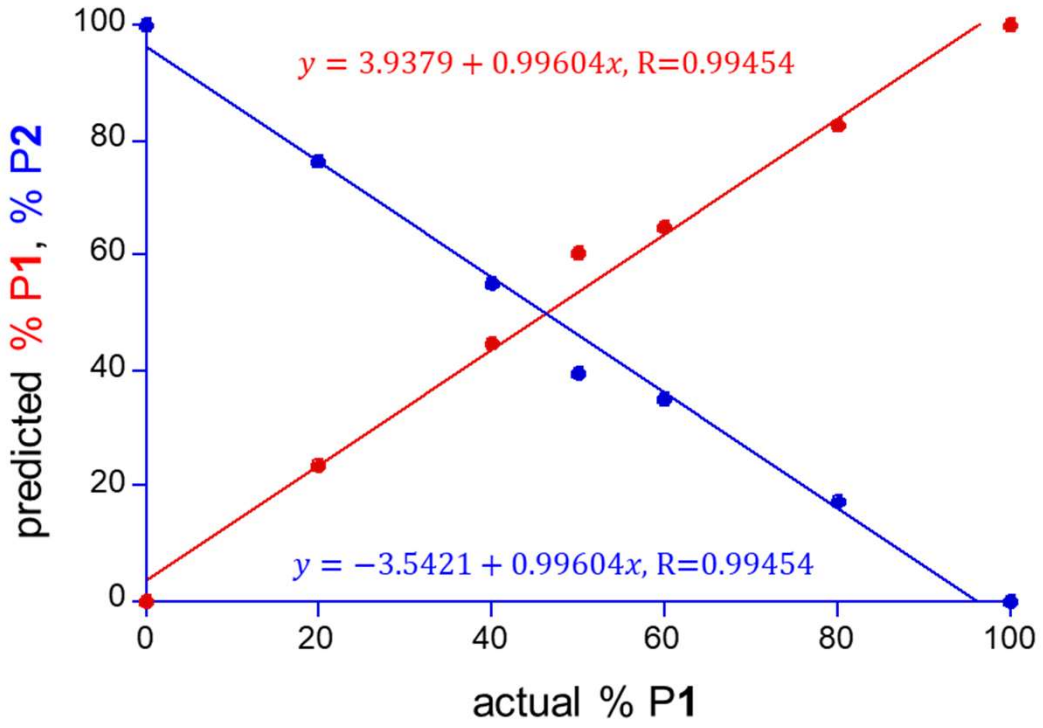


mixture inhomogeneity, signal shifting

LF Raman

co-added 6 spectra per mixture
direct classical least squares analysis
recovery curves

P1		P2	
Known	Predicted	Known	Predicted
0	0	100	100
20	23.58	80	76.42
40	44.67	60	55.33
50	60.45	50	39.55
60	64.9	40	35.1
80	82.58	20	17.42
100	100	0	0



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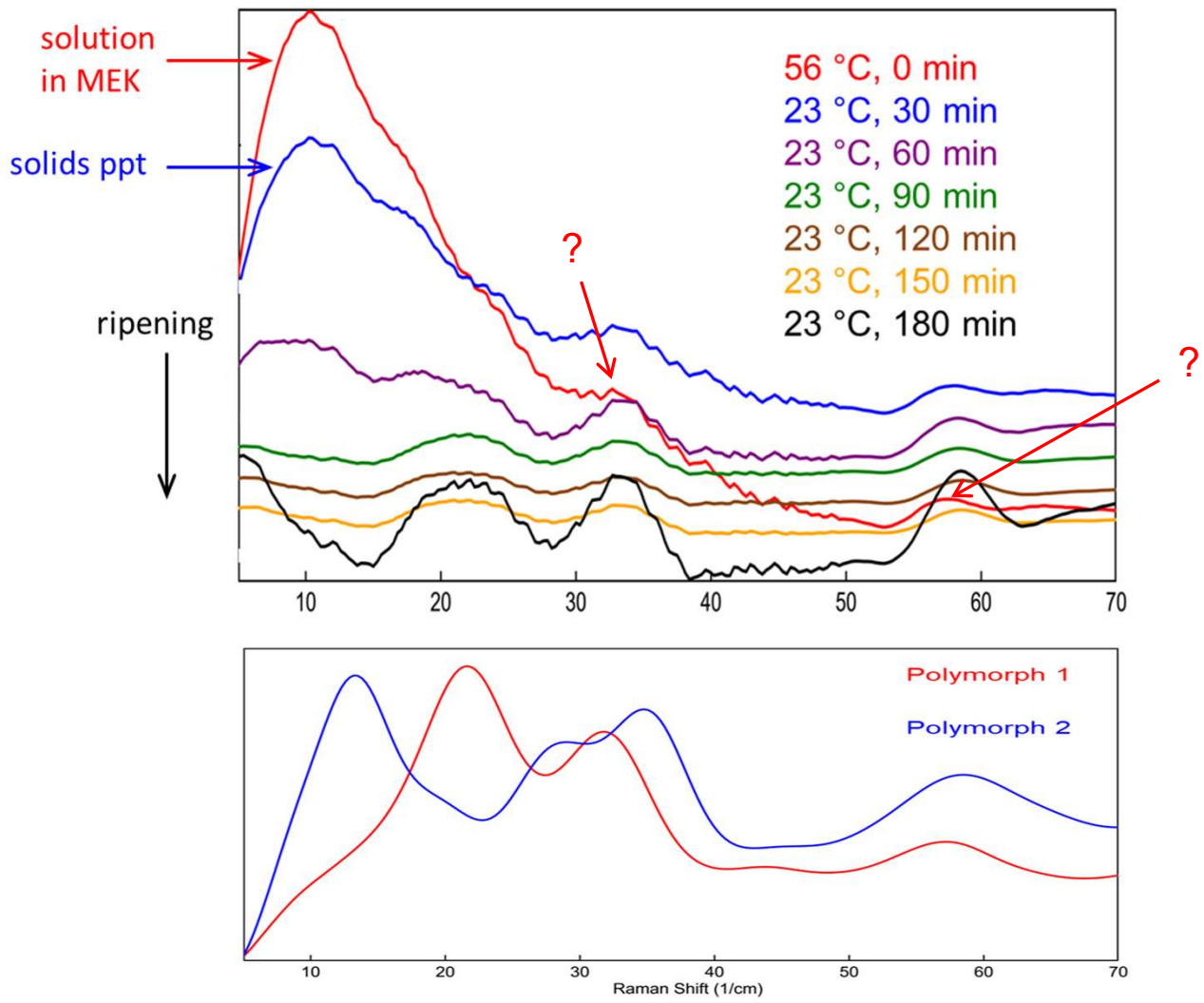
In-Situ Monitoring of Crystallization



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Additional LF Raman Experiments

- Solvent appears to drive polymorph: P**1** from MEK, P**2** from alcohols
  - Monitor in-situ crystallization from alcohols
  - Add turbidity probe and monitor crystallizations with both
- Re-run slurry interconversion experiment and monitor in-situ
  - Use data to quantify amounts of P**1** and P**2** throughout the process

Triclinic is routinely characterizing solid forms using LF Raman spectroscopy and considers it to be a viable technique for quantitative solid mixture analysis

Thank you for your attention

Questions welcome

Triclinic Labs, Inc.
2660 Schuyler Ave., Suite A
Lafayette, IN 47905 USA
765.588.6200
www.tricliniclabs.com